AQ

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : A61K 7/06 — A2

(11) International Publication Number: WO 96/09806

(43) International Publication Date: 4 April 1996 (04.04.96)

(21) International Application Number: PCT/US95/12134

(22) International Filing Date: 21 September 1995 (21.09.95)

(30) Priority Data:
314,327 28 September 1994 (28.09.94) US

(60) Parent Application or Grant
(63) Related by Continuation
US 314,327 (CON)
Filed on 28 September 1994 (28.09.94)

(71) Applicant *(for all designated States except US)*: HANDELMAN, Joseph, H. [US/US]; 26 West 61st Street, New York, NY 10023 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: AHLUWALIA, Gurpreet, S. [US/US]; 8632 Stable View Court, Gaithersburg, MD 20879 (US). SHANDER, Douglas [US/US]; 16112 Howard Landing Drive, Gaithersburg, MD 20878 (US). STYCZYNSKI, Peter [US/US]; 3709 Roop Road, New Windsor, MD 21776 (US).

(74) Agents: GALLOWAY, Peter, D.; Ladas & Parry, 26 West 61st Street, New York, NY 10023 (US) et al.

(81) Designated States: AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TT, UA, UG, US, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG), ARIPO patent (KE, MW, SD, SZ, UG).

Published
Without international search report and to be republished upon receipt of that report.

(54) Title: INHIBITION OF HAIR GROWTH

(57) Abstract

Mammalian hair growth is reduced by applying to the skin a composition including an inhibitor of protein kinase C.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	**GB**	United Kingdom	**MR**	Mauritania
AU	Australia	**GE**	Georgia	**MW**	Malawi
BB	Barbados	**GN**	Guinea	**NE**	Niger
BE	Belgium	**GR**	Greece	**NL**	Netherlands
BF	Burkina Faso	**HU**	Hungary	**NO**	Norway
BG	Bulgaria	**IE**	Ireland	**NZ**	New Zealand
BJ	Benin	**IT**	Italy	**PL**	Poland
BR	Brazil	**JP**	Japan	**PT**	Portugal
BY	Belarus	**KE**	Kenya	**RO**	Romania
CA	Canada	**KG**	Kyrgystan	**RU**	Russian Federation
CF	Central African Republic	**KP**	Democratic People's Republic of Korea	**SD**	Sudan
CG	Congo			**SE**	Sweden
CH	Switzerland	**KR**	Republic of Korea	**SI**	Slovenia
CI	Côte d'Ivoire	**KZ**	Kazakhstan	**SK**	Slovakia
CM	Cameroon	**LI**	Liechtenstein	**SN**	Senegal
CN	China	**LK**	Sri Lanka	**TD**	Chad
CS	Czechoslovakia	**LU**	Luxembourg	**TG**	Togo
CZ	Czech Republic	**LV**	Latvia	**TJ**	Tajikistan
DE	Germany	**MC**	Monaco	**TT**	Trinidad and Tobago
DK	Denmark	**MD**	Republic of Moldova	**UA**	Ukraine
ES	Spain	**MG**	Madagascar	**US**	United States of America
FI	Finland	**ML**	Mali	**UZ**	Uzbekistan
FR	France	**MN**	Mongolia	**VN**	Viet Nam
GA	Gabon				

INHIBITION OF HAIR GROWTH

The invention relates to a method of reducing unwanted hair growth in mammals, and to a cosmetic method for this purpose.

A main function of mammalian hair is to provide environmental protection. However, that function has largely been lost in humans, in whom hair is kept or removed from various parts of the body essentially for cosmetic reasons. For example, it is generally preferred to have hair on the scalp but not on the face.

Various procedures have been employed to remove unwanted hair, including shaving, electrolysis, depilatory creams or lotions, waxing, plucking, and therapeutic antiandrogens. These conventional procedures generally have drawbacks associated with them. Shaving, for instance, can cause nicks and cuts and can also promote the perception of an increase in the rate of hair regrowth. Shaving also can leave stubble. Electrolysis, on the other hand, can keep a treated area free of hair for prolonged periods of time, but can be expensive, painful, and sometimes leaves scarring. Depilatory creams, th ugh very effectiv , typically are not recommended for frequent use due t their high irritancy potential. Waxing and plucking can

cause pain, discomfort, and poor removal of short hair. Finally, antiandrogens -- which have been used to treat female hirsutism -- can have unwanted side effects.

It has previously been disclosed that the rate and character of hair growth can be altered by applying to the skin inhibitors of certain enzymes. These include inhibitors of 5-alpha reductase, ornithine decarboxylase, S-adenosylmethionine decarboxylase, gamma-glutamyl transpeptidase, and transglutaminase. See, for example, Breuer et al., U.S. Pat. No. 4,885,289; Shander, U.S. Pat. No. 4,720,489; Ahluwalia, U.S. Pat. No. 5,095,007; Ahluwalia et at., U.S. Pat. No. 5,096,911; Shander et al., U.S. Pat. No. 5,132,293; and Shander et al., U.S. Pat. No. 5,143,925.

It has now been found that unwanted mammalian (including human) hair growth -- particularly androgen-stimulated hair growth -- can be inhibited by applying to the skin a composition including a protein kinase C ("PKC") inhibitor in an amount effective to reduce hair growth. The unwanted hair growth which is reduced may be normal hair growth, or hair growth that results from an abnormal or diseased condition.

PKC is a phospholipid-dependent, calcium-sensitive family of enzymes that have the ability to phosphorylate proteins. PKC includes an ATP binding cite, a calcium binding site, and a region which interacts with phospholipid. Preferred inhibitors of PKC include those inhibitors that interact with one or more of these specific binding sites.

Among the inhibitors f PKC that can b used are (1) is quin line sulfonamides such

as 1-(5-isoquinolinyl sulfonyl)-2-methylpiperizine and its derivatives (J. Biol. Chem. 264:810-815, 1989); (2) bisindolylmaleimides such as 3-[1-(3-dimethylamino)propyl]-1H-indol-3-yl]-4-(1H-indol-3-yl)-1H-pyyrole-2,5-dione monohydrochloride (GF109203X), Ro 31-7549, and derivatives of Ro 31-7549 (Biochem. J. 294:335-337, 1993; J. Biol. Chem. 266:15771-15781, 1991; and J. Invest. Dermatol. 100:240-246, 1993); (3) phenothiazine derivatives such as thioridazine, trifluoperizine, and triflucarbine (J. Dermat. Sci. 4:18-25, 1992; and J. Biol. Chem. 255:8378-8380, 1980); (4) lysosphingolipids such as sphingosine and derivatives of sphingosine (Science 235:670-674, 1987; and Ann. Rev. Pharmacol. Toxicol. 32:377-397, 1992); (5) staurosporine, and derivatives of staurosporine such as 7-oxostaurosporine and 11-hydroxystaurosporine (Carcinogenesis 13:355-359, 1992; J. Antibiot. 45:195-198, 1992; and J. Org. Chem. 57:6327-6329, 1992); (6) verapamil, phentolamine and imipramine (J. Biol. Chem. 255:8378-8380, 1980); (7) L-ascorbic acid 6-palmitate (Cancer Res. 47:6633-6638, 1987); (8) glycyrrhetinic acid glycoside and 18β-glycyrrhetinic acid (Cancer Letters 49:9-12, 1990); (9) polymyxin B, sangivamycin, and doxorubicin-Fe(III) (J. Dermat. Sci. 4:18-25, 1992; J. Biol. Chem. 263:1682-1692, 1988; and Trends in Pharmacol. Sci. 12:188-194, 1991); (10) the fungal product, balanol, isolated from Verticillium balanoides (J. Am. Chem. Soc. 115:6452-6453, 1993); (11) substituted indolocarbazoles (Bioorg. Med. Chem. Let. 3:1959-1964, 1993); (12) 2-(aminomethyl)piperidines (J. Med. Chem. 34:2928-

2931, 1991); (13) curcumin (FEBS Lett rs 341:19-22, 1994); (14) 4-propyl-5(4-pyridinyl)-2-(3H)-oxazolone (Cancer Research 52:1195-1200, 1992); and (15) dequalinium (Trends in Pharmacol. Sci. 12:188-194, 1991). The inhibitors can be irreversible or reversible (competitive and non-competitive).

The inhibitor of PKC preferably is incorporated in a topical composition which includes a non-toxic dermatologically acceptable vehicle or carrier which is adapted to be spread upon the skin. Examples of suitable vehicles are acetone, alcohols, or a cream, lotion, or gel which can effectively deliver the active compound. One such vehicle is disclosed in co-pending application PCT/US 93/0506A. In addition, a penetration enhancer may be added to the vehicle to further enhance the effectiveness of the formulation.

The concentration of the inhibitor in the composition may be varied over a wide range up to a saturated solution, preferably from 0.1% to 30% by weight or even more; the reduction of hair growth increases as the amount of inhibitor applied increases per unit area of skin. The maximum amount effectively applied is limited only by the rate at which the inhibitor penetrates the skin. Generally, the effective amounts range from 100 to 3000 micrograms or more per square centimeter of skin.

The composition should be topically applied to a selected area of the body from which it is desired to inhibit hair growth. For example, the composition can be applied to the fac , particularly to the beard area of the face, i. ., the cheek, n ck, upp r lip, and chin. Th comp sition can also b applied to

the legs, arms, torso r armpits. The composition is particularly suitable for reducing the growth of unwanted hair in women suffering from hirsutism or other conditions. In humans, the composition should be applied once or twice a day, or even more frequently, for at least three months to achieve a perceived reduction in hair growth. Reduction in hair growth is demonstrated when the frequency of hair removal (shaving, tweezing, depilatory use, waxing) is reduced, or the subject perceives less hair on the treated site, or quantitatively, when the weight of hair removed by shaving (i.e., hair mass) is reduced. Benefits of reduced hair removal frequency include convenience and less skin irritation.

Male intact Golden Syrian hamsters are considered acceptable models for human beard hair growth in that they display oval shaped flank organs, one on each side, each about 8 mm. in major diameter, which grow thick black and coarse hair similar to human beard hair. These organs produce hair in response to androgens in the hamster. To evaluate the effectiveness of a particular PKC inhibitor, the flank organs of each of a group of hamsters are depilated by applying a thioglycolate based chemical depilatory (Surgex). To one organ of each animal 10-25 μl. of vehicle alone once a day is applied, while to the other organ of each animal an equal amount of vehicle containing a PKC inhibitor is applied. After thirteen applications (one application per day for five days a week), the flank organs are shaved and the amount of recovered hair (hair mass) from each is weighed. Percent-r ducti n of hair growth is calculated by subtracting the hair

mass (mg) valu f the test compound treated side from the hair mass value of the vehicle treated side; the delta value obtained is then divided by the hair mass value of the vehicle treated side, and the resultant number is multiplied by 100.

The above-described assay will be referred to herein as the "Golden Syrian hamster" assay. Preferred compositions provide an inhibition in hair growth of at least about 35%, more preferably at least about 50%, and most preferably at least about 70% when tested in the Golden Syrian hamster assay.

A number of PKC inhibitors were tested in the Golden Syrian hamster assay; the results are presented in Table I.

TABLE I

Compound	Vehicle[a]	Dose	pH	Hair Mass (means ± SEM) Untreated	Treated	%Inhibition
Verapamil	A	10%	5.5	1.45±.12	0.43±.05	69±5
Thioridazine	A	10%	4.5	2.41±.19	0.74±.07	68±4
Curcumin	C	10%	5.5	1.66±.19	0.55±.11	68±5
Trifluoperizine	A	10%	7.5	2.38±.25	1.03±.16	56±6
H-7[b]	A	10%	6.0	1.76±.18	0.81±.08	54±3
L-Ascorbic acid 6-palmitate	A	10%	8.5	2.82±.31	1.48±.14	46±5
Glycyrrhetinic acid glycoside	A	10%	4.0	1.84±.19	0.94±.17	46±10
18β-Glycyrrhetinic acid	B	7.5%	7.5	2.01±.22	1.07±.10	44±6
Imipramine	A	10%	5.5	1.52±.22	0.94±.18	38±5
Phentolamine	A	10%	6.0	2.05±.27	1.61±.22	19±9

[a]Vehicle A includes pure water (68%), ethanol (16%), propylene glycol (5%), dipropylene glycol (5%), benzyl alcohol (4%) and propylene carbonate (2%); vehicle B includes ethanol (80%), pure water (10%) and dipropylene glycol (10%); and vehicle C includes acetone (40%), ethanol (20%), DMSO (20%), and water (20%)

[b]H-7 is 1-(5-isoquinolinylsulfonyl)-2-methylpiperazine.

PKC activity was assayed in hair follicles isolated from flank organs using a commercial assay kit obtained from GIBCO BRL (Gaithersburg, MD). The assay is based on the phosphorylation (incorporation of ^{32}-P into) of acetylated-myelin basic protein. After isolation, the flank organ hair follicles were washed in phosphate-buffered saline and homogenized in a buffer containing 20 mM Tris, pH 7.5, 0.5 mM EDTA, 0.5% Triton X-100, 10 mM β-mercaptoethanol, and 25 μg/each of the protease inhibitors aprotinin and leupeptin. The hair follicle homogenate was added to the PKC reaction mixture at a final concentration of 10-20 μg/assay. The assay also included buffer, H_2O, phospholipid. The assay was performed in the presence or absence of select PKC inhibitors. The reaction mixture volume was 50 μl, the ^{32}P-ATP substrate was added in a volume of 10 μl. The reaction proceeded at 32° for 15 minutes, whereupon a 16.3 μL aliquot was removed from the reaction mixture and spotted onto a paper filter. Filters were washed twice in 1% phosphoric acid with gentle shaking for 5 minutes. Filters were then washed twice in H_2O and placed in scintillation vials. ^{32}P-Incorporation, a measure of the enzyme activity, was determined using standard liquid scintillation techniques. A significant inhibition was observed with thioridazine (30% inhibition at 500 μM) and trifluoperizine (42% inhibition at 500 μM) -- which are thought to interfere with the phospholipid binding site -- as well as with H-7, the most selective of PKC inhibitors and ATP binding site antagonist. An 86% inhibition of phosphorylati n due to inhibition of PKC activity was produced by 200

μM H-7. Inhibition of PKC activity was nearly 100% with glycyrrhetinic acid glycoside (glycyrrhizine) at 200 μM; and 52% with 18β-glycyrrhetinic acid at 500 μM.

It will be appreciated by those skilled in the art that the invention can be performed within a wide range of equivalent parameters of composition and conditions without departing from the spirit or scope of the invention or of any embodiment thereof.

CLAIMS

1. A method of inhibiting mammalian hair growth, comprising

selecting an area of skin from which reduced hair growth is desired; and

applying to said area of skin a composition of an inhibitor of protein kinase C in an amount effective to reduce hair growth.

2. The process of claim 1, wherein said inhibitor is verapamil.

3. The process of claim 1, wherein said inhibitor is thioridazine.

4. The process of claim 1, wherein said inhibitor is curcumin.

5. The process of claim 1, wherein said inhibitor is trifluoperizine.

6. The process of claim 1, wherein said inhibitor is 1-(5-isoquinolinylsulfonyl)2-methylpiperazine.

7. The process of claim 1, wherein said inhibitor is L-ascorbic acid 6-palmitate.

8. The process of claim 1, wherein said inhibitor is glycyrrhetinic acid glycoside.

9. The process of claim 1, wherein said inhibitor is 18β-glycyrrhetinic acid.

10. The process of claim 1, wherein said inhibitor is imipramine.

11. The process of claim 1, wherein said inhibitor is phentolamine.

12. The process of claim 1, wherein said inhibitor is an isoquinoline sulfonamide.

13. The process of claim 1, wherein said inhibitor is a bisindolylmaleimide.

14. The process of claim 1, wherein said inhibitor is a phen thiazine derivative.

15. The process of claim 1, wherein said inhibitor is a lysosphingolipid.

16. The process of claim 1, wherein said inhibitor is a staurosporine.

17. The process of claim 1, wherein said inhibitor is selected from the group consisting of polymyxin B, sangivamycin, and doxorubicin-Fe(III).

18. The process of claim 1, wherein said inhibitor is balanol.

19. The process of claim 1, wherein said inhibitor is a substituted indolocarbazole.

20. The process of claim 1, wherein said inhibitor is a 2-(aminomethyl)piperidine.

21. The process of claim 1, wherein said inhibitor is 4-propyl-5(4-pyridinyl)-2-(3H)-oxazolone.

22. The process of claim 1, wherein said inhibitor is dequalinium.

23. The process of claim 1, wherein said inhibitor interacts with the ATP binding site in PKC.

24. The process of claim 1, wherein said inhibitor interacts with the calcium binding site in PKC.

25. The process of claim 1, wherein said inhibitor interacts with the region in PKC which interacts with phospholipid.

26. The process of claim 1, wherein said inhibitor is an irreversible inhibitor.

27. The process of claim 1, wherein the concentration of said inhibitor in said composition is between 1% and 30%.

28. The process of claim 1, wherein the composition is applied to the skin in an amount of from 100 to 3000 micrograms of said inhibitor per square centimeter of skin.

29. The proc ss of claim 1, wherein the composition is appli d to the skin n the fac

f said mammal.

30. The process of claim 1, wherein the composition provides a reduction in hair growth of at least 30% when tested in the Golden Syrian hamster assay.

31. The process of claim 1, wherein the composition provides a reduction in hair growth of at least 50% when tested in the Golden Syrian hamster assay.

32. The process of claim 1, wherein the composition provides a reduction in hair growth of at least 70% when tested in the Golden Syrian hamster assay.

33. The process of claim 1, wherein said mammal is a human.

34. The use of an inhibitor of protein kinase C for the manufacture of a medicament for inhibiting mammalian hair growth.

35. The use according to claim 34, wherein said inhibitor is as defined in any one of claims 2-26.

36. A method of producing a composition for inhibiting mammalian hair growth, which comprises selecting an inhibitor of protein kinase C, and combining said inhibitor, in an amount effective to reduce hair growth, with a non-toxic, dermatologically acceptable vehicle or carrier.

37. A method according to claim 36, wherein said vehicle or carrier is adapted to be spread upon the skin of a mammal.

38. A method according to claim 36, wherein said inhibitor is as defined in any one of claims 2-26.

39. The new use of an inhibitor of protein kinase C for reducing hair growth.

40. A composition when used for inhibiting

mammalian hair growth, which includes an inhibitor of protein kinase C in an amount effective to reduce hair growth and a non-toxic, dermatologically acceptable vehicle or carrier.

41. A composition according to claim 40, wherein said inhibitor is as defined in any one of claims 2-26.